EXHIBIT B1

Postdoctoral research proposal: Growth Regulation of The Melanocyte

The direct contacts between melanocytes and keratinocytes are vital in maintaining the normal characteristics of melanocytes. Keratinocytes control melanocyte proliferation, production of melanin, and dendricity in vivo and when cocultivated in vitro with primary melanocytes . Contact with keratinocytes is not observed in transformed (oncogenic) melanocytes known as melanoma cells. Of paramount importance is the adhesion molecule E-cadherin, which links normal melanocytes and keratinocytes . Loss of E-cadherin in particular is implicated in tumor invasiveness in many cancers including melanoma, probably by reducing melanocyte-keratinocyte association. In addition, loss of E-cadherin in melanocytes is associated with expression of a new adhesion molecule known as N-cadherin, which may enhance invasion of the dermis by melanocytes. It is widely believed that inhibition of E-cadherin synthesis by melanocytes represents one of the earliest steps in development of melanoma and one that cannot be studied outside the context of epidermal architecture. Very little is currently understood of the regulation of melanocyte contact with keratinocytes, the signals which maintain this interaction, and how they are disrupted very early in development of melanoma. What is known is that loss of the intimate contact between dendritic melanocytes and normal keratinocytes is a primary event in the development of melanoma. Studies are proposed to begin to understand how keratinocytes regulate synthesis of E-cadherin in melanocytes.

A mechanism by which keratinocytes control melanocyte growth and dendrite formation is through synthesis and secretion of a mitogenic factor, endothelin-1. Melanocytes have high affinity receptors for endothelin-1, which induces melanocyte-specific tyrosinase activity (involved in melanin production), production of melanin and dendrite formation which is required for melanosome transfer to keratinocytes . Importantly, melanoma cells have greatly decreased numbers of endothelin-1 receptors, which is tightly correlated with loss of dendritic contact and invasiveness. We aim to elucidate the mechanism for loss of dendrite formation in melanocytes, particularly the role played by downregulation of endothelin-1 or endothelin receptors. It will be determined whether E-cadherin synthesis in melanocytes is dependent upon the presence of endothelin-1 or

endothelin receptors and whether the signal for new synthesis of N-cadherin is the absence of endothelin signaling.

There is intriguing evidence that the activity of neurofibromin, the product of the NF-1 gene, may also play a role in maintaining normal melanocyte function and that loss of NF-1 function may be involved in progression to melanoma. Expression of NF-1 in melanocytes stimulates tyrosinase gene expression (a marker for normal melanocyte function). NF-1 also inhibits growth and induces differentiation of melanoma cells in which NF-1 is not ordinarily expressed. What is not clearly known is whether NF-1 expression is required for normal melanocyte function, for dendrite formation and. for keratinocyte interaction. It is also not known whether NF-1 induces synthesis of E-cadherin and suppresses synthesis of N-cadherin. These are all questions which will be addressed in this proposal.

The goal of these studies is to understand, at a molecular level, the key early events which determine loss of normal melanocyte growth control, ultimately leading to development of melanoma. Importantly, these studies will be carried out within the context of human epidermal architecture, using co-cultures of primary melanocytes and keratinocytes that closely recapitulate the crucial cell-cell contacts which form the basis for normal melanocyte growth and which are lost as the earliest observable changes during melanoma. We will specifically determine how melanocyte expression of E-cadherin is affected by 1)blocking keratinocyte production of endothelin-1, 2) blocking binding of endothelin-1 to its receptor site on the melanocyte and 3) inhibition of melanocyte NF-1 expression. We will also investigate whether the malignant phenotype of melanoma cells can be reversed by introduction of exogenous genes expressing either NF-1 or the endothelin-1 receptor and whether E-cadherin expression can be restored by these gene products.